Novel Zinc Chelators Which Inhibit the Binding of HIV-EP1, a Zinc Finger Protein, to NFKB Recognition Sequence (BIOORGANIC CHEMISTRY - Bioactive Chemistry)

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Novel Zinc Chelators Which Inhibit the Binding of HIV-EP1, a Zinc Finger Protein, to NF-κB Recognition Sequence

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In order to manipulate the function of zinc finger proteins, several zinc-binding molecules comprising dimethylaminopyridine and histidine units have been prepared. NMR study showed that trityl and carboxyl groups contributed to increase zinc-binding capability of the chelators. These molecules exhibited remarkable inhibitory effect on the DNA binding of the human immunodeficiency virus type 1 enhancer binding protein HIV-EP1 which contains two C_2H_2 type zinc fingers. DNA-binding capability of HIV-EP1 was recovered by adding extra zinc, confirming the inhibition to be caused by the removal of zinc from HIV-EP1. This approach could be a novel strategy for the control and elucidation of biochemical processes.

Keywords: HIV-EP1 / Zinc Finger /Transdription factor /DNA-binding

Zinc finger proteins constitute a major group of transcription factor and play important roles in the gene expression. Our interest has been focused on a C_2H_2 type zinc finger protein HIV-EP1 which binds to DNA κB site (5'-GGGACTTTCC-3') present in the long terminal repeat of HIV provirus to activates the HIV-1 gene expression. It was thought that the function of this zinc protein could be modulated by ejecting the zinc and inhibition of HIV-EP1 would lead to the interference of the replication of AIDS virus. The objective of this study is to construct an efficient zinc-coordinating system which can abstract zinc from HIV-EP1 to inhibit DNA binding.

Previously we reported a metal-chelating system comprising a dimethylaminopyridine and histidine methyl ester **1** (1). We considered that the structure of the compound **1** could be modified so as to be an efficient zinc trapper. First we tried to introduce a trityl group into the imidazole in order to alter the chelating characteristics of the imidazolyl group. We also attempted to change the methyl ester groups of compound **1** into carboxyls. Thus, we prepared trityl and/or carboxyl derivatives **2-4** starting with 1 (2, 3).

Treatment of metal-free **1**, **2**, **3**, and **4** with

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Scope of research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, probing the DNA fine structure by various chemicals, studies on the DNA recognition of zinc-finger proteins, construction of artificial restriction enzyme, and model study on the cooperative mechanism of DNA binding by dimeric peptides. Also studied are the design and synthesis of functional molecules that effectively regulate the intracellular signal transduction or that applicable to fluorescence detection of DNA.

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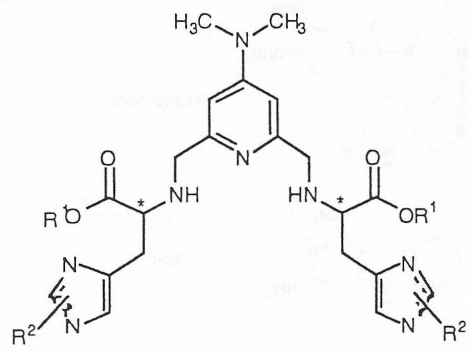
equimolar $ZnSO_4$ in MeOH afforded the corresponding 1:1 zinc complexes. Zinc-chelated **1**, **2**, **3**, and **4** thus formed were distinguished from the metal-free 1, 2, 3, and 4 by ^1H or ^{13}C NMR spectroscopy. The zinc-binding affinity of the synthetic ligands **2**, **3**, and **4** was compared with that of ligand 1 by competitive zinc binding expeiments using ^1H NMR. The affinity of the ligand 1 for zinc was decreased by introducing trityl group. However, zinc binding power was greatly improved by changing the methyl ester to the carboxyl, and *RS*-isomer **4** showed the highest affinity for zinc.



Compound	R^1	R^2	Stereochemistry (*)
1	CH_3	H	*SS*
2	CH_3	Trt	*SS*
3	H	Trt	*SS*
4	H	Trt	*RS*

The synthetic chelators **1-4** were found to exhibit remarkable inhibitory effect on the DNA binding of HIV-EP1, even more potent than that of EDTA, as demonstrated by electrophoretic mobility shift assay. The most potent was compound **1** which inhibited the DNA binding almost completely at 0.4 mM concentration. The most strong zinc chelator **4** showed somewhat weaker inhibition. Discrepancy between the DNA-binding inhibitory effect and the zinc-binding power of ligands may be due to their relatively low solubility in aqueous media or possibly due to the difference in the dissociation of the carboxyl group of the ligand depending on the solvent constitution (CD_3OD-D_2O (4:1) for the NMR measurement and H_2O-CH_3OH (96:4) for the DNA-binding experiments). All these ligands were shown to be stronger inhibitors of DNA binding compared with EDTA although EDTA showed stronger affinity for zinc, suggesting the superiority of the nitrogen-containing heterocyclic structure in terms of amino acid interaction, hydrophobic interaction, or possibly electronic effect favourable for the formation of presumed intermediary ternary complex with HIV-EP1-Zn. When zinc was introduced during or after the DNA-binding inhibition reaction with compound **1** (0.7mM), total recovery of HIV-EP1-DNA complex was observed. Ethidium displacement and footprinting experiments indicated that **1** has virtually no interaction with DNA. These indicated that the inhibition was indeed caused by the removal of zinc from the zinc finger moiety of HIV-EP1 and ruled out a competition between ligand **1** and HIV-EP1 for binding to DNA.

Thus, we developed novel zinc-binding heterocycles and succeeded in the inhibition of DNA binding of a zinc finger protein HIV-EP1. Since this approach can basically be applicable to any zinc proteins and the further structural modification of the pyridine-histidine system could be easily attained, the present study may provide a basis for the control and elucidation of various biochemical processes.

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